

```
$%^Other;HighlightOn=**;HighlightOff=**;  
Trying 11180...Open
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PLEASE ENTER HOST PORT ID:
PLEASE ENTER HOST PORT ID:x
LOGINID:d185jfr
PASSWORD:
TERMINAL (ENTER 1, 2, 3, 4, OR ?): 3
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Welcome to MESSENGER (APS Text) at USPTO
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The USPTO production files are current through:
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JANUARY 12, 1999 for U.S. Patent Text Data.
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JANUARY 12, 1999 for U.S. Current Classification Data.
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JANUARY 12, 1999 for U.S. Patent Image Data.
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* PLEASE USE 305-9000 FOR NEW TELEPHONE NUMBER *
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More U.S. patent data is now available on APS. The new
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USOCR file contains patents issued in 1970, plus some
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patents that were missing from the USPAT file. See the
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Patents News Folder under the Public Folders in e-mail for
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more information on using the new file. Thank you.
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DISCLAIMER:
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Neither the United States Government, nor any agency
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thereof, nor any of their contractors, subcontractors or
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employees make any warranty, expressed or implied,
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including any warranty of marketability of fitness for a
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particular purpose; nor assumes any legal liability or
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responsibility for any party's use, or the results of
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such, of the data.
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Help Desk --> 703-305-9000
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The Help Desk is staffed for APS support 7 days/week.
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Monday through Friday: 6:30am - 9:00pm
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Saturday, Sunday, Holidays: 8:30am - 5:00 pm
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The Help Desk staff at this number will handle all APS
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related questions.
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>>>>>>>>>> NEW SUNDAY HOURS !!! <<<<<<<<<<<
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The APS is available:
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6:30am - 9:00pm Monday through Friday
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7:30am - 5:00pm Saturday, Sunday, Holidays
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APS is unavailable Thanksgiving Day, Christmas Day,
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and New Year's Day.
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FILE "USPAT" ENTERED AT 16:19:52 ON 13 JAN 1999
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* * * * *
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WELCOME TO THE
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U.S. PATENT TEXT FILE
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* * * * *
```

⇒ s shigella

L1 2076 SHIGELLA

=> s mammal?

L2 48077 MAMMAL?

=> s 11(p)l2

L3 116 L1(P)L2

=> s entry or entered or enter

163758 ENTRY  
97439 ENTERED  
233513 ENTER

L4 385306 ENTRY OR ENTERED OR ENTER

=> s 14(p)l3

L5 3 L4(P)L3

=> d

1. 5,824,538, Oct. 20, 1998, Shigella vector for delivering DNA to a mammalian cell; Arthur A. Branstrom, et al., 435/252.1; 424/93.2; 435/245, 252.3, 455, 822 [IMAGE AVAILABLE]

=> d 1 pam

US PAT NO: 5,824,538 [IMAGE AVAILABLE] L5: 1 of 3

=> d fd pam

US PAT NO: 5,824,538 [IMAGE AVAILABLE] L5: 1 of 3  
DATE FILED: Sep. 6, 1995

=> d xa xp

US PAT NO: 5,824,538 [IMAGE AVAILABLE] L5: 1 of 3  
ASST-EXMR: Christopher R. Tate  
PRIM-EXMR: Leon B. Lankford, Jr.

=> d clms

US PAT NO: 5,824,538 [IMAGE AVAILABLE] L5: 1 of 3

CLAIMS:

CLMS(1)

What is claimed is:

1. A delivery vehicle for delivering a mammalian expression plasmid into a mammalian cell, said vehicle comprising a pure culture of attenuated Shigella cells into which said expression plasmid has been introduced, wherein said Shigella cells have at least one genetic mutation such that they lyse once inside said mammalian cell, thereby delivering said expression plasmid therein.

CLMS(2)

2. The delivery vehicle according to claim 1, wherein said Shigella is *S. flexneri*.

CLMS(3)

3. The delivery vehicle according to claim 1, wherein said mammalian cell is a cell of an intestinal mucosal epithelium.

CLMS(4)

4. The delivery vehicle according to claim 3, wherein said *Shigella* is *S. flexneri*.

CLMS(5)

5. The delivery vehicle according to claim 4, wherein said *S. flexneri* is strain 15D given ATCC accession number 55710.

CLMS(6)

6. The delivery vehicle according to claim 1, wherein said attenuated *Shigella* cells are inactivated.

CLMS(7)

7. The delivery vehicle according to claim 6, wherein said attenuated *Shigella* is heat-inactivated.

CLMS(8)

8. The delivery vehicle of claim 1, wherein said mutation is a mutation in the wild-type *asd* gene within said *Shigella* cells.

CLMS(9)

9. A method for delivering a mammalian expression plasmid into a mammalian cell, said method comprising:  
(i) introducing a mammalian expression plasmid into a pure culture of attenuated *Shigella* cells, wherein said *Shigella* cells have at least one genetic mutation such that they lyse once inside said mammalian cell; and  
(ii) administering said *Shigella* cells to said mammalian cell, thereby delivering said expression plasmid therein.

CLMS(10)

10. The method according to claim 9, wherein said *Shigella* is *S. flexneri*.

CLMS(11)

11. The method according to claim 10, wherein said *S. flexneri* is strain 15D, given ATCC accession number 55710.

CLMS(12)

12. The method according to claim 11, wherein said mammalian cell is a cell of a mucosal epithelium.

CLMS(13)

13. The method according to claim 9, wherein said mammalian cell is a cell of a mucosal epithelium.

CLMS(14)

14. The method according to claim 13, wherein said mucosal epithelium is intestinal mucosal epithelium.

CLMS(15)

15. The method according to claim 9, wherein said attenuated *Shigella* cells are inactivated.

CLMS(16)

16. The method according to claim 15, wherein said attenuated *Shigella* is heat-inactivated.

CLMS(17)

17. The method of claim 9, wherein said mutation is a mutation in the wild-type *asd* gene within said *Shigella* cells.

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(FILE 'USPAT' ENTERED AT 16:19:52 ON 13 JAN 1999)

L1 2076 S SHIGELLA  
L2 48077 S MAMMAL?  
L3 116 S L1(P)L2  
L4 385306 S ENTRY OR ENTERED OR ENTER  
L5 3 S L4(P)L3

=> s aspartate(4a)dehydrogenase

3866 ASPARTATE  
6789 DEHYDROGENASE  
L6 138 ASPARTATE(4A)DEHYDROGENASE

=> s l1 and l6

L7 9 L1 AND L6

=> d 1-4

1. 5,855,880, Jan. 5, 1999, Avirulent microbes and uses therefor; Roy Curtiss, III, et al., 424/93.2, 93.48, 184.1, 200.1, 235.1, 257.1, 258.1; 435/252.3, 252.33, 320.1, 879 [IMAGE AVAILABLE]
2. 5,855,879, Jan. 5, 1999, Avirulent microbes and uses therefor; Roy Curtiss III, 424/93.2, 93.48, 184.1, 200.1, 235.1, 257.1, 258.1; 435/252.3, 252.33, 320.1, 879 [IMAGE AVAILABLE]
3. 5,840,483, Nov. 24, 1998, Method of maintaining a desired recombinant gene in a genetic population of cells; Roy Curtiss, III, 435/6, 252.3, 252.33, 320.1 [IMAGE AVAILABLE]
4. 5,824,538, Oct. 20, 1998, **\*\*Shigella\*\*** vector for delivering DNA to a mammalian cell; Arthur A. Branstrom, et al., 435/252.1; 424/93.2; 435/245, 252.3, 455, 822 [IMAGE AVAILABLE]

=> d 4 fro

US PAT NO: 5,824,538 [IMAGE AVAILABLE] L7: 4 of 9  
DATE ISSUED: Oct. 20, 1998  
TITLE: **\*\*Shigella\*\*** vector for delivering DNA to a mammalian cell  
INVENTOR: Arthur A. Branstrom, Rockville, MD  
Donata R. Sizemore, Gaithersburg, MD  
Jerald C. Sadoff, Washington, DC  
ASSIGNEE: The United States of America as represented by the  
Secretary of the Army, Washington, DC (U.S. govt.)  
APPL-NO: 08/523,855  
DATE FILED: Sep. 6, 1995  
INT-CL: [6] C12N 1/00; C12N 1/20; C12N 15/00

US-CL-ISSUED: 435/252.1; 424/93.2; 435/172.1, 172.3, 245, 252.3, 822  
 US-CL-CURRENT: 435/252.1; 424/93.2; 435/245, 252.3, 455, 822  
 SEARCH-FLD: 424/234.1, 235.1, 93.2; 435/245, 172.3, 252.1, 252.3, 822,  
 172.1

REF-CITED:

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5,077,044	12/1991	Stocker	424/235.1
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Oaks et al. (1985) Plaque formation by virulent *Shigella flexneri*. *Infec. Immun.* 48:124-129.

Mills et al. (1988) *Shigella flexneri* invasion plasmid antigens B and C: epitope location and characterization with monoclonal antibodies. *Infec. Immun.* 56:2933-2941.

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Nakayama et al. (1988) Construction of an ASD.sup.+ expression-cloning vector: stable maintenance and high level expression of cloned genes in a *Salmonella* vaccine strain. *Bio/Technology* 6: 693-697.

Sansonetti et al. (1983) Alterations in the pathogenicity of *Escherichia coli* K12 after transfer of plasmid and chromosomal genes from *Shigella flexneri*. *Infec. Immun.* 39: 1392-1402.

Branstrom, Arthur A. (1993) Stable plasmid maintenance of HIV genes in *S. typhimurium* and *S. typhi*. Presented at the 33rd ICAAC, New Orleans, LA, 20 Oct. 1993, Abstract #1136.

Galan et al. *Gene*, vol. 94, pp. 29-35, 1990.

Hatten et al. *Gene*, vol. 129, pp. 123-128, 1993.

Lindberg et al. *Vaccine* vol. 6, pp. 146-150, 1988 Abstract Enclosed.

ART-UNIT: 161

PRIM-EXMR: Leon B. Lankford, Jr.

ASST-EXMR: Christopher R. Tate

LEGAL-REP: Charles H. Harris, John Francis Moran

ABSTRACT:

We describe a bacterial delivery system for the delivery of DNA and antigens into cells. We constructed an attenuated bacterial vector which enters mammalian cells and ruptures delivering functional plasmid DNA, such as a mammalian expression plasmid, and antigens into the cell cytoplasm. This **\*\*Shigella\*\*** vector was designed to deliver DNA to colonic surfaces, thus opening the possibility of oral and other mucosal DNA immunization and gene therapy strategies. The attenuated **\*\*Shigella\*\*** is also useful as a vaccine for reducing disease symptoms caused by **\*\*Shigella\*\***.

17 Claims, 11 Drawing Figures

=> d his

(FILE 'USPAT' ENTERED AT 16:19:52 ON 13 JAN 1999)  
 L1 2076 S SHIGELLA

L2 48077 S MAMMAL?  
L3 116 S L1(P)L2  
L4 385306 S ENTRY OR ENTERED OR ENTER  
L5 3 S L4(P)L3  
L6 138 S ASPARTATE(4A)DEHYDROGENASE  
L7 9 S L1 AND L6

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